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Oral pharmacological treatments for parasitic diseases of rainbow trout *Oncorhynchus mykiss*. I: *Hexamita salmonis*

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ABSTRACT: Various drugs were evaluated as regards efficacy for the treatment of *Hexamita salmonis* infection in rainbow trout. The results confirm the efficacy of nitroimidazoles: infection was completely eradicated not only by metronidazole (which has been recommended previously for the treatment of hexamitosis), but also by benznidazole, ronidazole and secnidazole, which have not been assayed previously. The non-nitroimidazoles albendazole, aminosidine, diethylcarbamazine and nitroscanate also completely eliminated infection. The remaining non-nitroimidazoles tested (amprolium, bithionol, febantel, flubendazole, levamisole, netobimin, niclosamide, nitroxynil, oxibendazole, parbendazole, piperazine, praziquentel, tetramisole, thiophanate, toltrazuril, trichlorfon and triclabendazole) were not effective.

KEY WORDS: Hexamitosis · Rainbow trout · Treatment Drugs

INTRODUCTION

Intestinal infection of fish by flagellates of the genus *Hexamita* is often associated with high mortality. Some authors have suggested that pathological effects arise when the host is weakened by other factors such as inadequate diet, a change in diet, low oxygen content in the water, overcrowding, inappropriate handling, and/or keeping fish of different sizes together (Becker 1977, Vickerman 1989). In both salmonids and tropical aquarium fish, intestinal parasitoses are often pathogenic only when the number of parasites present is very high (Post 1987 and Andrews et al. 1988, cited by Woo & Poynton 1995). Gratzek (1988) suggests that flagellate parasites interfere with nutrition by competing for essential nutrients and/or by damaging the intestinal epithelium.

Hexamitosis is probably the most frequent internal flagellate parasitosis of fish, notably in young salmonids, though also in carp, aquarium species, and various marine fish. Heavily infected fish are weak, listless, anorexic and emaciated, so that the head appears large with respect to the body ('pinhead fish'). Affected fish typically swim on their side, or with corkscrew movements (Lom & Dyková 1992, Woo 1994). Populations affected by acute hexamitosis show high mortality over a very short period, due to rapid multiplication of the parasite and associated damage to the intestinal epithelium. The chronic form of hexamitosis is likewise common, and generally occurs between spring and autumn; mortality per unit time is only slightly higher than in healthy fish, but severe losses may occur because the situation continues for a period of weeks (Woo 1994).

In trout and other salmonids infected with *Hexamita salmonis*, the effects commonly observed are anaemia, weight loss (Naich & Nilgees 1992), dark coloration, enteritis, excessive body mucus, and yellowish intestinal mucus (attributable to modified release of bile into the digestive tract); in addition, intestinal haemorrhage and liver cell necrosis may also be observed (Woo & Poynton 1995). Nevertheless, fish that appear to be heavily infected (i.e. numerous parasites in the pyloric caecae and the intestine) may show no signs of damage to the mucosa, and no evidence of invasion of the epithelium by the parasite (Ferguson 1979).

The only oral pharmacological treatments of *Hexamita salmonis* described to date have been drugs of the

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nitroimidazole group [Herwig et al. 1979, Reinhold et al. 1983 (cited by Woo 1984), Ghittino 1985, Lázaro-Chavez 1985, Imamovic 1986, Schmahl et al. 1989], which show activity against various protozoan groups, including flagellates and ciliates. The current treatment of choice is dimetridazole or metronidazole in the feed (Stoskopf 1993, Woo 1994, Woo & Poynton 1995).

There have been few studies of the possible anti-*Hexamita* activity of drugs of other groups. Here, we report a screening study of the possible efficacy of 21 non-nitroimidazole drugs at high doses (40 g per kg of feed for 10 d) for treatment of rainbow trout infected with *Hexamita salmonis*. As positive controls, we treated some fish with 1 of 5 nitroimidazoles, including metronidazole, as recommended for this parasitosis by Herwig et al. (1979) and Stoskopf (1993).

MATERIALS AND METHODS

Fish. Rainbow trout *Oncorhynchus mykiss*, Walbaum (weighing 16 g at the start of the experiments) were obtained from a local fish farm (Piscifactorías Coruñesas S.A., Carballo, A Coruña) and acclimatized for at least 36 h before assay in a 350 l tank with aeration and continuous flow of water (15 ± 3°C, pH 6.5 ± 0.5) from a nearby spring. Fish were fed daily with a commercial feed.

Infection. All fish used in the trials showed high-intensity infection by *Hexamita salmonis*, in some cases as a result of natural infections, and in other cases following experimental infection in the laboratory. Parasite-free fish were experimentally infected by holding them for 15 to 20 d in a 350 l tank that also contained fish showing high-intensity infection (400 uninfected fish to 100 infected fish). Twenty fish were then sampled at random for determination of infection intensity (see below). Since infection intensity was high in only 10 of the 20 fish, the experimental infection period was extended by 7 d, after which infection intensity was again determined in 10 randomly sampled fish. At this time all 10 fish showed high-intensity infection, and the assays were thus commenced.

Determination of infection intensity. Fish were anaesthetized by bathing in MS-222 (Sandoz; 0.05 g l^{-1}) until respiration became weak. A faecal sample was then obtained by gently pressing the abdomen. The sample was mixed with 3 drops of water on a slide, coverslipped and examined with a light microscope (400×). Estimation of infection intensity was recorded on a 5-point scale, after examination of a sample area of 24 × 32 mm, as follows: 'zero' (–), *Hexamita salmonis* not detected in the sample; 'minimal' (+/–), only 1 individual of *H. salmonis* detected in the sample; 'low' (+), more than 1 individual of *H. salmonis* detected in the sample, the average number per microscope field being less than 10; 'moderate' (++), average number of individuals per microscope field 10 to 50; 'high' (+++), average number of individuals per microscope field >50.

Drugs and assay design. The drugs tested in the study are listed in Table 1. Each drug was assayed in an 80 l tank containing 20 infected fish. A simultaneous control assay (also of 20 fish; identical treatment, but without drug) was performed for each drug. Tank conditions (water source, flow, aeration, pH, temperature, light/dark cycle) were identical to those during the acclimatization period. The treated fish received either (1) feed containing 40 g kg^{-1} of drug (non-nitroimidazoles) for the 10 d, or (2) feed containing 2 or 5 g kg^{-1} of drug (nitroimidazoles) for 2 d. Unamended feed was administered to fish of control group. In all cases feed was supplied at 2% of total body weight per day. Throughout the assay period the fish were monitored regularly to ensure that they were eating the food, and to check for signs of toxicity. Twenty-four hours after the end of the assay, fish were anaesthetized for determination of infection intensity as above.

Table 1. Drugs used in the study, showing manufacturer, brand and form of presentation. p.p. = pure product. *Nitroimidazoles

Drug	Brand name	Presentation	Manufacturer
Albendazole	p.p.	Powder	Ovejero
Aminosidine	Gabbrocol	Injectable	Vetern S.P.A.
Amprolium	Prolsal	Powder	Iteve
Benznidazole	p.p.	Powder	Roche
Bithionol	p.p.	Powder	Syva
Diethylcarbamacine	p.p.	Powder	Cidan
Febantel	p.p.	Powder	Bayer
Flubendazole	p.p.	Powder	Esteve
Levamisole	p.p.	Powder	Ovejero
Metronidazole*	Flagyl	Powder	Rhone Mérieux
Netobimin	p.p.	Powder	Ovejero
Niclosamide	Fugotenil	Pills	Uriach
Nitroscanate	Lopatol 500	Pills	Ciba-Geigy
Nitroxynil	p.p.	Powder	Ovejero
Oxibendazole	p.p.	Powder	Syva
Parbendazole	p.p.	Powder	Smith kline
Piperazine	Piperso l	Granules	Sobrino
Praziquentel	Droncit	Pills	Bayer
Ronidazole*	p.p.	Powder	Sobrino
Secnidazole*	p.p.	Powder	Rhone Mérieux
Tetramisole	p.p.	Powder	Ovejero
Tiophanate	p.p.	Powder	Uriach
Toltrazuril	p.p.	Powder	Bayer
Trichlorfon	Neguvón	Powder	Bayer
Triclabendazole	p.p.	Powder	Ciba-Geigy

Table 2. Results of the assays with non-nitroimidazoles that proved ineffective. For each drug (in all cases administered at 40 g per kg feed for 10 d), 24 h post-treatment infection intensity (as determined by faecal examination; see text) is shown for each of the 20 fish included in the assay for that drug. Infection intensity: +++, high; ++, moderate; +, low; +/–, minimal; –, zero (i.e. no *Hexamita salmonis* detected in faeces). nd: not determined; D: fish died during experiment

Drug	Trout number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Amprolium	+++	++	++	++	++	+	+	+	+	+/–	+/–	+/–	–	–	–	–	–	–	–	–
Bithionol	++	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	nd	nd
Febantel	+++	+++	+++	++	++	+	+	+	+/–	–	–	–	–	–	–	–	–	–	–	–
Flubendazole	+++	+	+	+	+/–	+/–	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–
Levamisole	+++	+++	+++	+++	++	++	++	+	+	+	+	+/–	–	–	–	–	–	–	–	–
Netobimin	+++	++	++	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Niclosamide	+++	++	++	++	++	+	+	+	+	+/–	–	–	–	–	nd	nd	nd	nd	nd	nd
Nitroxynil	+++	+++	++	++	++	+	+	+	–	–	–	–	–	–	–	–	–	–	nd	D
Oxibendazole	++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Parbendazole	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Piperazine	+++	+++	+++	+	+	+	+/–	–	–	–	–	–	–	–	–	–	–	nd	D	D
Praziquentel	+++	+++	++	–	–	–	–	–	–	–	–	–	–	–	–	–	nd	nd	nd	nd
Tetramisole	+++	+++	+++	+++	++	++	++	++	+	+	+	+	+/–	–	–	–	–	–	–	–
Tiophanate	+++	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Toltrazuril	+++	+++	+++	++	++	++	++	++	++	+	+	+	+	+/–	–	–	–	–	–	–
Trichlorfon	+/–	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Triclabendazole	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	nd	nd	nd

RESULTS

Of the 21 non-nitroimidazoles, 17 were not effective (i.e. did not completely eliminate infection); the results for these drugs are listed in Table 2. The results for the 4 effective non-nitroimidazoles (albendazole, aminosidine, diethylcarbamazine, nitroscanate) are listed in Table 3; all 4 eliminated infection in all 20 fish included in each assay. None of the 4 effective non-nitroimidazoles had evident toxic effects.

The 4 nitroimidazoles likewise completely eliminated infection at 5 g per kg feed for 2 d (Table 4). All nitroimidazoles except metronidazole were effective even at the lower dose of 2 g per kg feed for 2 d (Table 4). These results confirm the efficacy of these drugs when administered for a shorter period and at much lower doses than the other drugs tested.

DISCUSSION

In the present study we considered a wide range of anti-parasitic drugs, including 3 that have been recommended for the treatment of protozoan parasitoses of fish, namely amprolium (Awakura & Kurahashi 1967, Taylor et al. 1973, Herwig et al. 1979, Schmahl et al.

Table 3. Results of the assays with non-nitroimidazoles that proved effective. For each drug-treated group (drug administered at 40 g per kg feed for 2 d) and the corresponding control group, 24 h post-treatment infection intensity (as determined by faecal examination; see text) is shown for each of the 20 fish included in that assay. Infection intensity: +++, high; ++, moderate; +, low; +/–, minimal; –, zero (i.e. no *Hexamita salmonis* detected in faeces). nd: not determined; D: fish died during experiment

Drug	Trout number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Abendazole	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	–	–	–	–	–	D
Aminosidine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	nd
Control	+++	+++	+++	+++	++	++	++	++	++	+	+	+	+	+	+	+	+/–	–	–	nd
Diethylcarbamacine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control	+++	++	++	++	++	++	+	+	+	+	+/–	+/–	+/–	–	–	–	–	–	–	–
Nitroscanate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	nd	nd
Control	+++	+++	+++	+++	+++	+++	++	++	–	–	–	–	–	–	–	–	–	–	–	nd

Table 4. Results of the assays with the 4 nitroimidazoles. For each drug-treated group (drug administered at 2 or 5 g per kg feed, for the first 2 d of the 10 d experimental period) and the corresponding control group, post-treatment infection intensity (as determined by faecal examination; see text) is shown for each of the 20 fish included in that assay. Infection intensity: +++, high; ++, moderate; +, low; +/–, minimal; –, zero (i.e. no *Hexamita salmonis* detected in faeces). nd: not determined

Drug		Trout number																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Benznidazole	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control	–	+++	+++	+++	+++	+++	+++	++	++	++	++	+	–	–	–	–	–	–	–	–	–
Metronidazole	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control	–	+++	+++	+++	+++	++	++	++	++	++	+	+	+	+	+	+	+	+/–	–	–	nd
Ronidazole	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control	–	+++	+++	+++	+++	+++	+++	++	++	++	++	+	–	–	–	–	–	–	–	–	–
Secnidazole	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Control	–	+++	+++	+++	+++	+++	+++	++	++	++	++	+	–	–	–	–	–	–	–	–	–

1989, Stoskopf 1993), bithionol (Tojo 1993) and toltrazuril (Melhorn et al. 1988, Schmahl & Melhorn 1989a, b, Schmahl et al. 1989, Schmahl et al. 1990, Yokoyama et al. 1990, Schmahl et al. 1991, Stoskopf 1993).

Of these, toltrazuril is that which has been most widely used for the treatment of parasitoses of fish, and indeed this drug has been recommended for the treatment of various microsporidian and myxosporidian infections (Schmahl & Melhorn 1989a, b). This drug is not, however, effective for the treatment of infestation by the ectoparasitic ciliate *Ichthyophthirius multifiliis* (From et al. 1992, Tojo et al. 1994c).

Of these drugs (amprolium, bithionol and toltrazuril), the only one previously found to be effective after oral administration is amprolium; indeed, its administration by this route is recommended for myxosporidiosis. Bithionol and toltrazuril have been shown to be effective only in bath treatments. In a study of efficacy for the treatment of infestation by the flagellate *Ichthyobodo necator*, complete elimination of infestation in all fish assayed was achieved after bathing with bithionol at 25 mg l^{-1} for 3 h on 2 consecutive days, but not after bathing with amprolium or toltrazuril (Tojo et al. 1994a).

In the present study, none of these drugs was effective for oral treatment of *Hexamita salmonis* infection, despite the fact that the dose used was much higher than in similar previous studies. Equally ineffective were febantel, flubendazole, levamisole, netobimin, niclosamide, nitroxynil, oxibendazole, parbendazole, piperazine, praziquentel, tetramisole, thiophanate, trichlorfon and triclabendazole; this is in accordance with previous studies, none of which has demonstrated antiprotozoan activity of these drugs in fish (Obermeier 1974, Griffin 1989, Rydlo 1989, Tojo et al. 1994a, b).

The only non-nitroimidazole drugs that completely eliminated infection were albendazole, aminosidine, diethylcarbamazine and nitroscanate. Of these, the only one previously recommended for the treatment of infection by *Hexamita salmonis* is aminosidine (15 g per kg feed for 3 consecutive days) (Herwig et al. 1979). Nitroscanate appears to have a rather broad activity spectrum, since it has been shown to be effective for bath treatment of *Gyrodactylus* (Santamarina et al. 1991). Neither albendazole nor diethylcarbamazine has previously been shown to be effective for treatment of protozoan parasitoses of fishes (see Griffin 1989).

Four nitroimidazoles were used in the present study as positive controls. Nitroimidazoles are currently the only drugs recommended for oral treatment of *Hexamita salmonis* infection. We used doses of 2 or 5 g per kg feed for 2 d. Previous reports have recommended dimetridazole at 1.5 g per kg feed for 3 d (Herwig et al. 1979), or at 15 g per kg feed for 4 to 7 d (Schmahl et al. 1989). Metronidazole has been recommended at doses of 0.5 mg per kg feed for 2 d (Imamovic 1986), 20 mg per kg feed for 2 d (Reinhold et al. 1983, cited by Woo 1994), or 1.5 g per kg feed for 3 d (Lázaro-Chavez 1985). Bath treatment with metronidazole (at 4 mg l^{-1}) has been recommended for infections with *Trichodina*, *Ambiphyra* and *Chilodonella* (Moore et al. 1984). Dimetridazole and metronidazole have also been tested against other protozoan parasitoses (bath treatment against *Ichthyobodo necator*; oral and bath treatment

against *I. multifiliis*; Tojo 1993), with negative results. As far as we are aware, the other nitroimidazoles tested in the previous study (benznidazole, ronidazole and secnidazole) have never been used for the treatment of *H. salmonis* infection.

Dimetridazole was not included in our assays, since we have previously found it to be toxic in bath treatment (Tojo 1993). Oral treatment of trout with some nitroimidazoles has been reported to affect behaviour (Tojo 1993). The nitroimidazoles used here were selected in view of the absence of evident toxic effects when administered orally (Tojo 1993). Likewise, none of these drugs showed negative effects (signs of toxicity, behavioural effects including anomalous swimming movements, rejection of food) in the present study, suggesting that all 4 nitroimidazoles assayed are viable options for the treatment of infection of salmonids by *Hexamita salmonis*. This is of particular interest in view of the fact that *H. salmonis* strains apparently resistant to metronidazole have recently appeared on some farms (authors' unpubl. obs.).

Finally, it is worth noting that *Hexamita salmonis*-infected fish pass both trophozoites and cysts in faeces (Lom & Dyková 1992, Woo 1994). However, in the present study, as in related previous studies, infection was detected on the basis on the presence of trophozoites in faeces; trophozoites are readily detected and identified by their characteristic movement, even at low magnification. Cysts were not observed at any stage, even after subjecting samples to concentration methods such as the Bailinger method. Previous studies have likewise failed to detect cysts in the faeces of infected fish (Kulda & Lom 1964, Kent et al. 1992). Cysts thus appear to be very rare.

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